Gene Expression Patterns in Near Isogenic Lines for Wheat Rust Resistance Gene *Lr34/Yr18*

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ABSTRACT

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The *Lr34/Yr18* resistance gene provides durable, adult-plant, slow rusting resistance to leaf rust, yellow rust, and several other diseases of wheat. Flag leaves may exhibit spontaneous leaf tip necrosis and tips are more resistant than leaf bases. Despite the importance of this gene, the mechanism of resistance is unknown. Patterns of expression for 55,052 transcripts were examined by microarray analysis in mock-inoculated flag leaves of two pairs of wheat near isogenic lines for *Lr34/Yr18* (Jupateco 73S/Jupateco 73R and Thatcher/Thatcher-*Lr34*). The Thatcher isolines were also examined for patterns of expression after inoculation with leaf rust. Mock-inoculated leaf tips of resistant plants showed up-regulation of 57 transcripts generally associated with ABA inducibility, osmotic stress, cold stress, and/or seed maturation. Several transcripts may be useful as expression markers for *Lr34/Yr18*. Five transcripts were also up-regulated in resistant leaf bases. The possible role of these transcripts in resistance is discussed. In mock-inoculated plants, pathogenesis-related (PR) proteins were not up-regulated in resistant flag leaves compared with that in susceptible flag leaves. In inoculated plants, the same set of PR proteins was up-regulated in both resistant and susceptible flag leaves. However, expression was often higher in resistant plants, suggesting a possible role for *Lr34/Yr18* in priming of defense responses.

Additional keywords: green island effect, race nonspecific resistance, stripe rust.

Yield stability of wheat is constantly challenged by a number of biotic and abiotic stress factors. Rust diseases, including leaf rust (*Puccinia triticina* Eriks.), yellow or stripe rust (*P. striiformis* Westend.), and stem rust (*P. graminis* Pers.:Pers.), are globally the most important foliar diseases of wheat. Genetic resistance is the preferred method for controlling all three rusts and dozens of resistance genes have been reported for each rust species (22,33). However, wheat is cultivated in huge acreages which provide many opportunities for emergence of new pathotypes that can overcome the resistance genes deployed by breeders. The break-down of resistance in popular cultivars leaves few options in regions where fungicide treatments are not possible or economically feasible. In addition, the deployment of new resistance genes is a long process, often relying on introgression from related species. Consequently, most wheat improvement programs are seeking more durable resistance strategies to protect against rust diseases.

For typical resistance genes, the mechanism of resistance and the basis of race specificity are due to gene-for-gene interactions between host resistance genes (*R*-genes) and pathogen avirulence (*Avr*) genes. Factors thought to influence durability of such resistance include the size and genetic plasticity of pathogen populations (31), effects of gene combinations or pyramids (43), the physiological cost of losing the corresponding *Avr* gene for the pathogen (2,26), and the level of selection pressure conferred by the resistance gene (27). Various strategies have been proposed to prolong the usefulness of *R*-genes (31).

Some disease resistance genes, like the *mlo* powdery mildew resistance gene of barley, have nonspecific effects and are apparently durable because they are not dependent on the recognition of a single *Avr* gene product from the pathogen (38,59). A few rust resistance genes have emerged among the many studied that appear to be associated with nonspecific durable resistance. The most well-known of these is the *Lr34/Yr18* wheat rust resistance gene (12,22).

Several characteristics distinguish *Lr34/Yr18* from many other rust resistance genes. First, its effect is nonspecific; that is, no rust pathotypes have been shown to be completely virulent on lines carrying *Lr34/Yr18* (13,22,51). Rubiales and Niks (42) reported that *Lr34/Yr18* conditions prehaustorial resistance and is associated with reduced intercellular hyphal development but not with a hypersensitive response or papilla formation. It increases the latent period, decreases the number of pustules per unit area, and decreases pustule size as components of a slow rusting phenotype (10,42,50). These effects are greater at temperatures below 25°C, and the expression of resistance in flag leaf apices is greater than in leaf bases. Although it is considered an adult-plant gene, subtle effects of *Lr34/Yr18* can also be detected at younger growth stages (48,50). The effect of *Lr34/Yr18* is additive or synergistic with other minor or major genes (15,51).

The most unusual characteristic of this gene is its apparent pleiotropism. It confers adult-plant resistance to both leaf rust and stripe rust (32,46) and these two effects have never been separated by recombination (53). The *Lr34/Yr18* locus has also been associated with tolerance or resistance to other diseases, including *Barley yellow dwarf virus* (*Bdv1*, 47), powdery mildew (53), and stem rust (12,30). *Lr34/Yr18* is also completely linked with a gene conferring leaf tip necrosis in some environments (*Ltn1*; 45,49).

The peculiar characteristics of *Lr34/Yr18* strongly suggest that it conditions an unusual mechanism of resistance. Based on fine mapping studies, Bossolini et al. (4) suggested the possible in-

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volvement of a pectate lyase gene in resistance conferred by *Lr34/Yr18*. Rosewarne et al. (41) associated a β1-proteasome subunit with resistance conferred by *Lr46/Yr29*, another wheat slow rusting gene (52). The broad spectrum of resistance and lack of race specificity are reminiscent of systemic acquired resistance (SAR) associated with expression of pathogenesis-related (PR) proteins (57). The leaf tip necrosis and higher resistance of leaf tips are suggestive of accumulation of autotoxic and/or antimicrobial compounds. Considering the increasing dependence on *Lr34/Yr18* and other slow rusting genes for durable rust resistance (51), it is essential to obtain a greater understanding of its mechanism of resistance.

The present analysis was initiated to identify gene expression patterns and possible defense processes regulated by *Lr34/Yr18* using microarray analysis. We used two pairs of near isogenic lines for *Lr34/Yr18* (Thatcher/Thatcher-*Lr34* and Jupateco 73S/Jupateco 73R) to help reduce genetic background effects unrelated to *Lr34/Yr18*. We tested both inoculated and mock-inoculated flag leaves to identify possible induced and constitutive defense mechanisms. We examined flag leaf tips and bases separately because leaf tips are more resistant than bases and leaf tips often display necrosis in *Lr34/Yr18* plants. We report evidence that *Lr34/Yr18* is associated with expression of a set of abiotic stress and/or seed maturation genes not usually associated with pathogen defense reactions.

MATERIALS AND METHODS

Plant material. Two pairs of spring habit near isogenic lines (hereafter referred to as isolines) for *Lr34/Yr18* were used: Thatcher/Thatcher-*Lr34* and Jupateco 73S/Jupateco 73R. Thatcher-*Lr34* (R.L.6058) containing *Lr34/Yr18* was derived from PI 58548 backcrossed six times to Thatcher (12). Jupateco 73S (without *Lr34/Yr18*) and Jupateco 73R (with *Lr34/Yr18*) were near isogenic selections from the Mexican cv. Jupateco 73 (49,50).

The Thatcher and Jupateco isoline pairs were grown 6 months apart as separate experiments. For each isoline pair, the experiment was a factorial design with two factors (resistant/susceptible isoline and inoculated/mock-inoculated) and three replications. Experimental units were single plants grown in 4-liter pots in pasteurized silt-loam/peat/coarse perlite soil mix (12:55:3, vol/vol/vol) with 2% (wt/wt) gypsum. Pots were watered daily to maintain soil moisture near field capacity. Plants were grown in a 36 ft^2 growth chamber (Conviron PGW-36) under 15°C night/ 20°C day (±1°C) with a 16 h photoperiod and approximately 500 μmol m^{-2} s^{-1} photosynthetically active radiation at the top of the canopy. Relative humidity varied from approximately 70% during the day to 90% at night. Pots were fertilized at planting with 5 g of 14-14-14 Osmocote (The Scotts Company, Marysville, OH) per pot and thereafter every 2 weeks with 0.5 g of 15-30-15 Miracle-Gro All Purpose Plant Food per pot (Scott's Miracle-Gro Products, Inc.) and 0.1 ml of Fertilome Chelated Liquid Iron and Other Micronutrients (Voluntary Purchasing Groups, Inc., Bonham, TX) per pot. No pest problems were detected during the experiments and no pesticides were applied.

Thatcher isolines were uniformly and heavily inoculated with the *P. triticina* race MFBL (see literature citation 23 for race descriptions) suspended in Soltrol 170 light oil (Chevron Phillips Chemical Company, The Woodlands, TX). Thatcher and Thatcher-*Lr34* do not contain effective major genes against this race. Mock-inoculated plants were sprayed only with Soltrol oil. Inoculation was done at 6:00 p.m., and the inoculated and mock-inoculated plants were then incubated overnight in a mist chamber in a greenhouse at 20 ± 3°C with 100% relative humidity for 14 h. The plants were returned to the growth chamber until sampling at 9:00 a.m. on the third day after inoculation, which was 48 h after removal from the mist chamber.

One or two flag leaves were harvested from tillers of single plants at the anthesis stage (Feekes stage 10.51 to 10.53). Necrotic and chlorotic portions of the leaf tip were carefully excised and discarded. The apical half (hereafter referred to as tip) and the basal half (hereafter referred to as base) of the leaves were handled separately and immediately frozen with liquid nitrogen and stored at –80°C. Each isoline–treatment combination had three biological replications. After sampling was completed, unsampled tillers of mock-inoculated plants were inoculated with a virulent race and the expected resistance phenotypes were verified. Jupateco isolines were inoculated with race MQNS, which was chosen to overcome *Lr17*.

RNA isolation and microarray procedure. Frozen leaf tissue was ground in liquid nitrogen and immediately suspended in TRIzol reagent, and total RNA was isolated following the manufacturer's suggested protocol (Invitrogen, Carlsbad, CA). Isolated RNA was further purified with Qiagen (Valencia, CA) RNeasy columns. Five micrograms of total RNA was used for first-strand cDNA synthesis using Affymetrix (Santa Clara, CA) One-Cycle Target Labeling and Control Reagent. Second-strand cDNA and cRNA synthesis/labeling were performed using the same kit following the manufacturer's instructions. Fifteen micrograms of cRNA was fragmented and used for chip hybridization. RNA quality was tested using an Agilent Bioanalyzer 2100 (Agilent Technologies, Palo Alto, CA).

GeneChip Wheat Genome Array. The GeneChip (Affymetrix) Wheat Genome Array contains 61,127 probe sets representing 55,052 transcripts predicted to be unique. Each probe set is made up of 11 matching and 11 mismatching 25-mer oligonucleotides that provide multiple independent estimates of signal and background hybridization levels. Probe sets with different numbers after the decimal usually denote related unigenes. Alphabetic suffixes denote whether probe sets may contain probes that are perfect matches to more than one exemplar. Some transcripts may be monitored by more than one probe set and some probe sets may match more than one transcript. The sequence information comes from publicly available expressed sequence tag (EST) sequences from wheat (*Triticum aestivum*) and wheat relatives (*T. monococcum*, *T. turgidum*, and *Aegilops tauschii*). The available wheat sequences included more than 34,000 ESTs from libraries made from tissues treated with high or low temperatures or stressed by drought or salt treatment. They also included more than 42,000 sequences from tissues treated with various plant pathogens. *T. aestivum* expression controls include ubiquitin, 18S rRNA, glucose-6-phosphate dehydrogenase, cytochrome P450 CYP86, sucrose synthase, actin, elongation factor-1α, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

The Thatcher experiment used a total of 24 microarray chips. However, only 12 microarray chips were available for the Jupateco experiment and therefore only the mock-inoculated tissue was used. The allocation of all 36 microarray chips is described in Table 1.

Data analysis. After alignment of the image settings for each chip, raw data were extracted and marked as "present" (well above the background), "marginal", or "absent" (similar to, or below the background) under default settings. The average signal intensity of each chip was scaled to 500 and analyzed using GeneSpring GX 7.3.1 software (Agilent Technologies). The data were prenormalized to the 50th percentile per chip and then normalized based on per-gene normalization with the median method, from which the signal of each gene in a particular sample was divided by the median signal of the same gene in all samples. Microarray data were deposited in the NCBI Gene Expression Omnibus (available online from the National Center for Biotechnology Information [NCBI] website) under accession nos. GSM143490 through GSM143526.

Data from the Jupateco isolines, mock-inoculated Thatcher isolines, and inoculated Thatcher isolines were analyzed separately

for the effect of resistance on gene expression. Comparisons were made between samples from the same tissue type. For each probe set, one-way analysis of variance (ANOVA) was performed with three biological replicates to test the effect of resistant versus susceptible isolines. Fold changes were calculated as resistant divided by susceptible values. Probe sets were retained that were statistically significant ($P \leq 0.05$), showed greater than 1.5-fold change, and those that were marked as present by the GeneSpring software in at least two of six chips involved in a given comparison.

Data from the Thatcher-*Lr34* and Thatcher isolines were analyzed separately for the effect of inoculation on gene expression. Comparisons were made between samples from the same tissue type. Fold changes were calculated as inoculated divided by mock-inoculated values. Probe sets were retained that were statistically significant ($P \leq 0.05$), showed greater than a fivefold change, and those that were marked as present by the software in at least two of six chips involved in a given comparison.

Initial annotations of the probe sets on the arrays were performed using HarvEST software (provided online by the University of California). This provided a consensus sequence for each transcript (unigene) that was assembled from ESTs that were predicted to belong to that transcript. Each unigene sequence was used to search for homologous sequences of the GenBank NR and EST databases by both nucleotide (blastn) and predicted protein (tblastx). Predicted unigenes with regions of low quality sequence data were scrutinized to determine if they may represent chimeric sequences. If so, the distribution of probes on ESTs was used to identify the best homologs for annotation purposes. Sequences with significant matches to predicted proteins from other species with no known function were designated as predicted proteins and those with no significant matches ($P > 1e\text{-}05$) were considered as having no homology to characterized genes.

RESULTS

Rust resistance phenotypes. Inoculated flag leaves showed no symptoms at the time of sampling for RNA extraction at 48 h after removal from the mist chamber. Some flag leaves of resistant lines showed some leaf tip necrosis, but the necrosis was confined to the extreme tips. The first chlorotic flecks associated with rust infection were observed on susceptible lines at approximately 72 h after removal from the mist chamber. The first erupted pustules were observed on susceptible lines at approximately 120 h after removal from the mist chamber.

At 9 days after inoculation (192 h after removal from the mist chamber), inoculated flag leaves of resistant lines showed no rust

development and flag leaves of susceptible lines showed heavy development of leaf rust (Fig. 1). Resistant lines showed significant leaf tip necrosis in both the inoculated and mock-inoculated treatments. A few Thatcher flag leaves showed slight leaf tip necrosis.

At 15 days after inoculation, the apical halves of the flag leaves of resistant lines showed highly resistant reactions while the basal halves showed a moderate level of resistance; uredinia were fewer, smaller, and slower to develop than in the susceptible isolines (Fig. 1). Leaf tip necrosis on resistant plants was more pronounced in both the inoculated and mock-inoculated treatments at day 15 than at day 9. A few susceptible flag leaves showed slight leaf tip necrosis. A few mock-inoculated flag leaves of Thatcher isolines showed a few leaf rust pustules due to contamination during the inoculation process.

Microarray data quality. High quality data were obtained from all 36 microarray chips. Scaling factors ranged from 3.5 to 6.9 with an average of 4.9, and percent present calls ranged from 39.2 to 44.3% with an average of 41.6%. Noise ranged from 1.3 to 2.2 and averaged 1.7. *T. aestivum* control transcripts that showed the most stable signal intensities across treatments in the experiment were actin (Fig. 2A), elongation factor-1α, GAPDH, and ubiquitin (Fig. 2B).

Fig. 1. Resistance phenotypes of inoculated or mock-inoculated Thatcher-*Lr34* (Tc34) and Thatcher at 9 and 15 days after inoculation.

TABLE 1. Allocation of wheat microarray chips to mock-inoculated or inoculated leaf tips or leaf bases of resistant or susceptible isolines in Thatcher or Jupateco backgrounds

Cultivar background	Inoculation treatment	Leaf tissue[a]	Isoline[b]	Microarray chips[c]
Thatcher	Mock-inoculated	Tip	Resistant	3
			Susceptible	3
		Base	Resistant	3
			Susceptible	3
	Inoculated	Tip	Resistant	3
			Susceptible	3
		Base	Resistant	3
			Susceptible	3
Jupateco	Mock-inoculated	Tip	Resistant	3
			Susceptible	3
		Base	Resistant	3
			Susceptible	3

[a] Tip indicates the apical half of the flag leaf during anthesis; base indicates basal half.

[b] Resistant isolines contain *Lr34/Yr18* rust resistance gene.

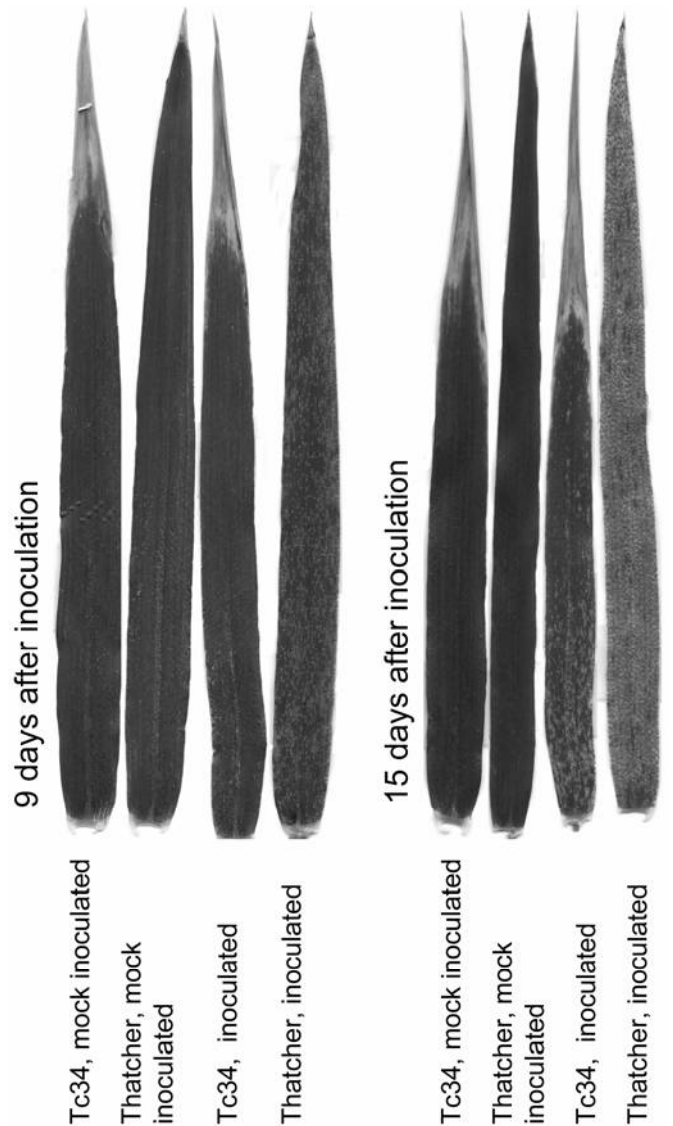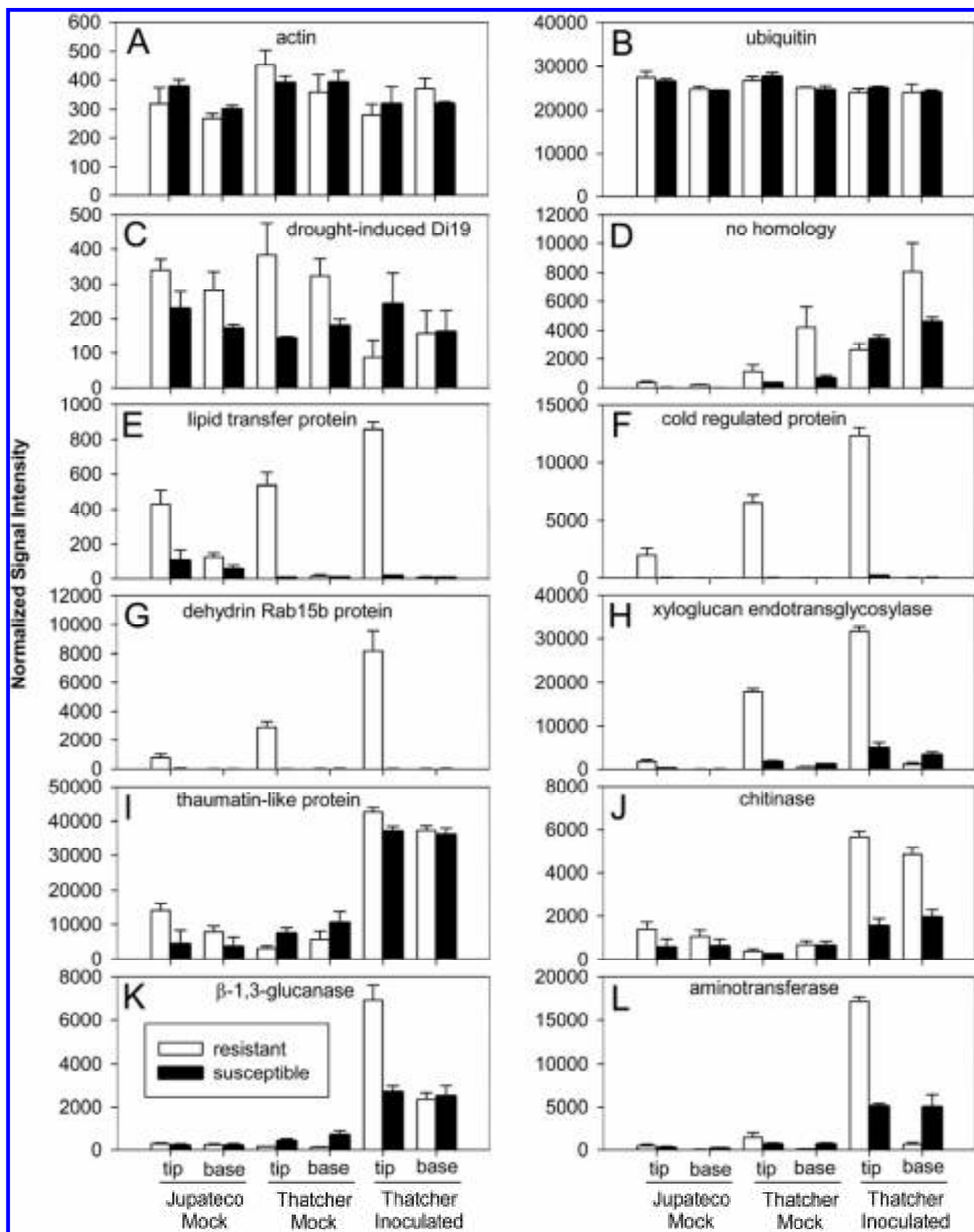[c] Each microarray chip was hybridized to separate biological replicates.

Fig. 2. Normalized signal intensities of Affymetrix Wheat GeneChip probe sets in tips or bases of flag leaves of mock-inoculated Jupateco isolines, mock-inoculated Thatcher isolines, and rust-inoculated Thatcher isolines with *Lr34/Yr18* (white bars) or without *Lr34/Yr18* (black bars). **A,** AFFX-Ta-actin-M_at; **B,** AFFX-Ta_Ubiquitin_3_at; **C,** Ta_6567.3.A1_x_at; **D,** Ta_14836.1.A1_at; **E,** Ta_28327.A1_at; **F,** Ta.13183.1.S1_s_at; **G,** Ta.2638.1.S1_at; **H,** Ta.13337.1.S1_at; **I,** Ta.959.1.S1_at; **J,** Ta.2278.1.S1_x_at; **K,** Ta.8584.1.S1_at; and **L,** TaAffx.21593.1.S1_at. Error bars represent standard error of the mean of three biological replications.

Genes up-regulated in mock-inoculated *Lr34/Yr18* lines. Comparisons of gene expression in the mock-inoculated resistant and susceptible isolines showed evidence of extensive differences in flag leaf gene expression without pathogen challenge. The number of genes that showed a significantly higher ($P \leq 0.05$ and at least 1.5-fold change) level of expression in *Lr34/Yr18* isolines was calculated for the tip and base for each of the two isoline pairs. In the tips of the Thatcher isoline flag leaves, 829 genes showed significantly more transcript in Thatcher-*Lr34* than in Thatcher and 163 showed at least fivefold more transcript. In contrast, only 470 genes were up-regulated more than 1.5-fold in the Thatcher-*Lr34* line in the basal half of the leaf and only 11 were up-regulated more than fivefold. A similar trend was observed in the Jupateco isolines but fewer genes were significantly affected in this experiment. There were 299 genes that were at least 1.5-fold more highly expressed in the leaf tips with only 33 showing more than fivefold higher expression. Only 149 and 17 showed more than 1.5- or fivefold higher expression in the leaf bases, respectively.

To reduce the false discovery rate attributable to experimental error or imperfect isogenicity of the isolines, the intersection of the sets of significant transcripts from mock-inoculated Thatcher and Jupateco isolines was examined. Fifty-seven transcripts were up-regulated at least 1.5-fold in the resistant leaf tips of both the Thatcher and Jupateco isolines, while only seven transcripts were consistently up-regulated in both experiments in the leaf bases (Table 2). Data from inoculated plants were not used for data filtering in this table, but are presented for comparison. The genes that were consistently up-regulated in resistant mock-inoculated leaves showed very similar patterns of up-regulation in inoculated resistant leaves (Table 2).

Many of the transcripts with increased expression in resistant leaf tips putatively encode stress-related proteins (Table 2, Fig. 2C to H). These included transcripts associated with desiccation, like dehydrins, and other proteins involved in seed maturation. Other gene products associated with osmotic stress due to drought or salt include pyrroline-5-carboxylate synthetase which is involved in proline synthesis (40,60), a putative galactinol synthase which is involved in synthesis of raffinose family oligosaccharides (7,62), and a gene with homology to the drought-induced *Arabidopsis* Di19 (17) protein. Other genes encoded proteins induced by cold stress or acclimation. These include genes found to be cold-regulated in barley (Ta.13183.1 [8]) or wheat (Ta.13380.1 [14]; TaAffx.80153.1 [56]; Ta.4222.1 [19]). Also up-regulated was the Ta.129.1 gene that codes for the ABA inducible plasma membrane protein PM19, which is also associated with cold tolerance (21), and another very closely related gene, Ta.14259.1. Other up-regulated transcripts represented a variety of metabolic processes, some of which are probably stress-related. These include transcripts for two proteinases and a proteinase inhibitor, and two xyloglucan endotransglycosylases, which are likely involved in cell wall modification. Possibilities for up-regulated regulatory or signaling proteins include three lipid transfer proteins (LTPs), three transcription factors, and a protein phosphatase 2C-like transcript.

Genes down-regulated in mock-inoculated *Lr34/Yr18* lines. Only a few genes were consistently down-regulated in mock-inoculated tissues and they were typically down-regulated only approximately twofold (Table 3). Only the Ta.12091.1 transcript was found to be down-regulated in both the Thatcher and Jupateco isolines in both the tips and bases of the leaves. The function of this gene is unknown. Five other transcripts were down-regulated in the mock-inoculated leaf bases but not the leaf tips. The CPRD2-like FAD-linked oxidoreductase and two xyloglucan endotransglycosylases were down-regulated in the bases but up-regulated in the leaf tips.

Changes in gene expression induced by rust inoculation. The effect of rust inoculation on gene expression in Thatcher and

Thatcher-*Lr34* in leaf tips and leaf bases was examined by comparing inoculated and mock-inoculated treatments. In the Thatcher-*Lr34* leaf tips, there were 1,478 genes that appeared significantly different ($P < 0.05$) and at least twofold higher expression in rust-infected tissue than in mock-inoculated tissue. Similarly, there were 1,610 genes with twofold higher expression in the rust-infected Thatcher leaves. In the basal halves of the leaves, 425 appeared significantly ($P < 0.05$ and twofold) rust-induced in the Thatcher-*Lr34* leaves and 610 in the Thatcher leaves. Because of the large numbers of genes examined, a more stringent criterion was used to create a list of rust-induced genes (Table 4), including only genes that were significantly up-regulated and showed at least a fivefold difference in at least three of the four tissues examined (leaf base or tip in Thatcher-*Lr34* or Thatcher). The resulting list of putative rust-induced transcripts contained 59 unigenes (Table 4) including typical PR proteins such as chitinases, glucanases, thaumatin-like proteins, genes involved in detoxification like cytochrome P450s, and genes involved in phenylpropanoid biosynthesis.

Only two of the transcripts found to be up-regulated in mock-inoculated resistant lines with *Lr34/Yr18* (Table 2) were among the most highly up-regulated by rust infection (Table 4), including a predicted protease inhibitor (TaAffx.132498.1) and a predicted aminotransferase (TaAffx.21593.1, Fig. 2L), which is probably GABA transaminase. Several of the other *Lr34/Yr18*-associated genes appeared moderately up-regulated by rust infection, showing at least twofold induction over the mock-inoculated control tissues in at least three of the four tissues examined (data not shown). These include one of the predicted dehydrins (Ta.13255.1), a xyloglucan endotransglycosylase (Ta.13337.2), a glutamate dehydrogenase (Ta.1870.1), two aldehyde dehydrogenases, (Ta.2107.1 and Ta.2107.3), an aldo/keto reductase (Ta.26922.1), a beta-D-glucan glucohydrolase (Ta.27751.5), and two unknown proteins (Ta.14224.1 and Ta.14836.1).

Both resistant and susceptible flag leaves expressed essentially the same set of up-regulated PR proteins and defense-related proteins after inoculation (Table 4). On average, the difference in response between inoculated and mock-inoculated tissues was 2.9-fold higher (*t* test, $P < 0.0001$) in the Thatcher-*Lr34* leaf tips and 2.1-fold higher (*t* test, $P < 0.0001$) in Thatcher-*Lr34* leaf bases than in Thatcher (Table 4). However, this broad view overlooks the fact that several different patterns of expression were exhibited by the transcripts in Table 4. One group was strongly rust-responsive but was not affected significantly by resistance. This group included Ta.959.1 (thaumatin-like protein, Fig. 2I), Ta.2784.1 (chitinase), Ta.21342.1 (chitinase), as well as TaAffx.83275.1, TaAffx.83275.3, Ta.27327.1, and TaAffx.31943.1 (all PR-1 proteins). Another group was strongly rust-responsive, but resistant leaf tips and bases responded much more strongly. This group included Ta.2278.1 (chitinase, Fig. 2J), Ta.2278.2 (chitinase), Ta.2278.3 (chitinase), Ta5810.1 (Wali6/Wali3-like), and Ta.8653.1 (no homology). A third group was most strongly rust-responsive in resistant tips. This group consisted of Ta.8584.1 (glucanase, Fig. 2K), Ta.24475.1 (glucanase), Ta.2690.1 (glutamine-dependent asparagine synthetase), and TaAffx.128418.43 (chitinase). The fourth group was similar, but seemed to be up-regulated in resistant tips and down-regulated in resistant leaf bases. It contained Ta.21593.1 (aminotransferase, Fig. 2L), TaAffx.108909.1 (aminotransferase), and Ta.22693.2 (predicted protein).

Two genes were highly down-regulated by rust infection using the same criterion as for the up-regulated genes. One transcript showed homology to cysteine proteinases (Ta.29534.1). The other down-regulated transcript (TaAffx.100446.1) was predicted to code for ACC oxidase (Table 4).

Wheat chemically induced genes. Five genes were previously identified as being highly induced by SAR-inducing chemicals in wheat (16). Treatment with benzothiadiazole (BTH) provided

TABLE 2. Genes up-regulated in resistant isolines with *Lr34/Yr18* in both Thatcher and Jupateco backgrounds

Probe set (unigene) designation[a]	Fold differences between *Lr34* and *lr34* transcript levels						Description
	Jupateco mock-inoculated		Thatcher mock-inoculated		Thatcher rust-inoculated		
	Tip[b]	Base	Tip	Base	Tip	Base	
Ta.2638.1.S1_at	34.5[c]		288.2		224.1		Dehydrin/Rab15b protein
Ta.13255.1.S1_at	20.9		33.8		15.4		Dehydrin
TaAffx.128555.1.S1_at	3.3		6.9		16.1		Dehydrin
TaAffx.131747.1.S1_x_at	13.51		2.2		3.9	1.6	Dehydrin
Ta.26049.1.S1_a_at[d]	2.2		21.8		86.1		Dehydrin
Ta.23797.1.S1_x_at	6.1		62.1		51.7		Late embryogenesis abundant protein (LEA)
Ta.28533.1.S1_at	8.2		15.0		19.2		Late embryogenesis abundant protein (LEA)
Ta.24158.1.S1_a_at	3.2		4.9		6.3		Embryo-specific protein
Ta.9389.1.S1_x_at	30.0		57.3		23.6		Soybean seed maturation protein, PM41
Ta.9389.1.S1_at	36.4		34.8		83.6	1.9	Soybean seed maturation protein, PM41
Ta.7091.1.S1_at	4.8		17.9		34.2		Pyrroline-5-carboxylate synthetase
TaAffx.17365.2.A1_at	4.6		2.2				Galactinol synthase
Ta.6567.3.A1_a_at	1.6	1.6	2.5	1.8			Drought-induced Di19 protein
Ta.14259.1.S1_at	11.3		28.5		86.2		ABA-induced plasma membrane protein
Ta.129.1.S1_at	3.1		3.3		4.7		ABA-induced plasma membrane protein PM 19
Ta.13183.1.S1_s_at	54.8		193.7		109.9		Cold-regulated protein, phosphatidylethanolamine-binding
Ta.13183.1.S1_x_at	14.6	1.7	26.2	1.9	58.0		Cold-regulated protein, phosphatidylethanolamine-binding
Ta.13380.1.S1_at	2.3		9.3		12.7		Low-temperature-specific wheat gene tacr7
TaAffx.80153.1.S1_at	1.9		5.7				Ice recrystallization inhibition protein
Ta.4222.1.S1_at	2.6		3.3		4.8		Low-temperature induced membrane protein
Ta.19158.1.A1_at	1.9		8.2		5.7		Subtilisin-like proteinase
Ta.3993.1.S1_at	2.3		6.8		8.7		Nucellain- or legumain-like protease
TaAffx.132498.1.S1_at	1.9		3.9		2.5		Maize protease inhibitor
Ta.11025.1.A1_at	3.0		62.5		50.9		CPRD2-like FAD-linked oxidoreductase
Ta.11623.2.A1_at	1.7		2.8		4.3		BTB/POZ domain-containing protein
Ta.13070.1.S1_at	2.8		3.2		3.3		Type 1 nonspecific lipid transfer protein
Ta.13070.2.S1_a_at	2.6		4.4		5.5		Type 1 nonspecific lipid transfer protein
Ta.28327.1.S1_x_at	8.4	3.5	99.9	1.6	56.2		Type 2 nonspecific lipid transfer protein
Ta.13337.1.S1_at	10.3		12.1		6.8		Xyloglucan endotransglycosylase
Ta.13337.2.S1_at	12.8		23.6		14.3		Xyloglucan endotransglycosylase
Ta.13337.2.S1_x_at	9.0		24.5		14.9		Xyloglucan endotransglycosylase
Ta.13682.1.A1_at	4.5		25.7		24.5		Progesterone 5-beta-reductase
Ta.1870.1.S1_a_at	2.7		3.3		2.2		Glutamate dehydrogenase
Ta.2107.1.S1_s_at	3.9		10.6		3.9		Aldehyde dehydrogenase
Ta.2107.3.S1_at	1.9		6.1		4.7		Aldehyde dehydrogenase
Ta.10207.1.S1_at	2.6		2.8		5.7		Protein phosphatase 2C-like
TaAffx.36680.1.A1_at	2.7		2.4				Transcriptional activator
TaAffx.122104.1.S1_at	3.0		5.0		5.8		NAC domain transcription factor
TaAffx.98394.1.S1_at	2.6		9.8		15.7		C2H2-type zinc finger protein
Ta.26922.1.S1_at	2.2		4.1		4.8		Aldo/keto reductase family
Ta.27751.5.S1_at	3.5		7.6		3.1		Beta-D-glucan glucohydrolase
Ta.28012.1.S1_a_at	2.0		1.7				Potassium transporter
Ta.29508.1.S1_at	1.7		2.0		1.7		Branched-chain alpha-keto acid dehydrogenase subunit
Ta.29508.3.S1_a_at	1.5		2.3		2.3		Branched-chain alpha-keto acid dehydrogenase subunit
Ta.8227.1.S1_at	2.3		2.5		2.9		Cytochrome P450
TaAffx.21593.1.S1_at	10.9		2.2		3.2		Aminotransferase
TaAffx.35563.1.A1_at	2.7		14.9		27.9		Peptide chain release factor subunit 1
Ta.5421.1.A1_at		1.7		1.6			Cytochrome oxidase assembly protein
TaAffx.4531.2.S1_at	2.3		1.7				Probable membrane protein
Ta.12896.1.S1_at	1.6		4.4		4.8		Predicted protein
Ta.29464.1.A1_at	5.4		2.1		2.3		Predicted protein
Ta.1327.1.S1_x_at	3.0		5.6		3.9		Predicted protein
TaAffx.12097.1.A1_at	2.7		17.5		16.0		Predicted protein
TaAffx.130713.1.A1_at	4.1		4.1		2.5		Predicted protein
TaAffx.64112.1.S1_at	1.7	2.1	2.9	4.4			Predicted protein
TaAffx.79120.2.A1_at	2.0		1.7				Predicted protein
Ta.14224.1.S1_at	3.0		4.2		4.4		Predicted protein
Ta.14836.1.A1_at	25.3	10.6	2.5	7.4		1.7	No homology
Ta.16163.1.S1_at	2.0		2.3				No homology
Ta.8866.2.S1_at	2.9		2.3		1.9		No homology
Ta.9831.1.S1_at	1.8		1.8				No homology
TaAffx.102050.1.S1_at		1.9		1.6			No homology

[a] Probe set designations from Affymetrix. Data include two different probe sets for the Ta.9389.1, Ta.13183.1, and Ta.13337.2 genes.

[b] Tip indicates the apical half of the flag leaf during anthesis; base indicates basal half.

[c] Indicates the fold change in *Lr34/Yr18* carrying lines compared with their respective susceptible isolines. Fold change for each cultivar background, inoculation treatment, and leaf tissue was calculated separately with three replicates. Only changes of ≥1.5 fold and $P ≤ 0.05$ are shown.

[d] Unigene Ta.26049.1 appears to be chimeric. Description based on portion of unigene sampled by probe set.

powdery mildew resistance and dramatically increased transcription of the wheat chemically induced genes, WCI-1 to WCI-5. When the transcript levels of these genes were examined in the present experiments, none of the genes was found to be significantly up-regulated by *Lr34* in mock-inoculated tissues (Table 2) and expression of three of the five was not significantly up-regulated by rust inoculation in the Thatcher isolines (Table 4). The WCI-5 gene was up-regulated by rust inoculation 2.1- and 2.7-fold in leaf tips of Thatcher-*Lr34* and Thatcher, respectively. The WCI-3 gene, predicted to encode a sulfur-rich, thionin-like protein, was more highly expressed (≈14-fold) in rust-infected leaf tips of Thatcher-*Lr34* than the corresponding tissues in Thatcher.

DISCUSSION

We used the Affymetrix Wheat GeneChip microarray to investigate the gene transcript profiles conferred by the durable, slow rusting resistance gene, *Lr34/Yr18*. We believe this is the first report using the Wheat GeneChip to study host–parasite interactions. In general, reproducibility was excellent among biological replicates with these microarrays (Fig. 2). The high correlation of expression data from the GeneChip microarray platform with northern blots or the serial analysis of gene expression method has already been documented (18,58). With 61,127 probe sets derived from 211 different EST libraries, transcriptome coverage is excellent. We concentrated on mock-inoculated leaves because previous studies indicated that resistance is prehaustorial and leaf tip necrosis occurs spontaneously in resistant leaves. By using two pairs of near isogenic lines, we were able to focus on transcripts that are reproducibly associated with resistance. By separately examining expression in leaf proximal halves (bases) and distal halves (tips), we were able to distinguish large differences in transcript abundance within portions of the same leaves. In addition to providing a baseline of transcript profiles for isolines with *Lr34/Yr18*, this data set is useful for generating and evaluating hypotheses about the mechanisms of resistance for this gene.

Patterns of gene expression of resistant isolines with *Lr34/Yr18* revealed a characteristic abiotic stress expression signature, which was observed in both mock-inoculated and rust-inoculated plants. Most members of this set of 57 up-regulated genes have been previously associated with abscisic acid (ABA) inducibility, drought/osmotic stress, cold stress, and seed maturation (Table 2). Most of these transcripts were highly up-regulated in the flag leaf tips, but not the leaf bases. This pattern correlates with the resistance phenotype since leaf tips are much more resistant than bases (Fig. 1). Typical PR proteins such as chitinases, glucanases, thaumatin-like proteins, and genes involved in phenylpropanoid biosynthesis were notably absent from the list of up-regulated transcripts in mock-inoculated leaves (Table 2).

Many of the signature transcripts showed quantitative differences between isolines, but several showed a qualitative expression pattern with little or no detectable transcript in susceptible plants. These include Ta.2638.1 (dehydrin/Rab15b protein, Fig. 2G), Ta.13183.1 (cold regulated protein, Fig. 2F), Ta.9389.1 (soybean seed maturation protein PM41), and Ta.13255.1 (dehydrin). Ta.28327.1 (LTP, Fig. 2E) and Ta.13337.1 (xyloglucan endotransglycosylase, Fig. 2H) show similar patterns, but they occasionally show low-level expression in susceptible plants. Ta.2638.1 and Ta.13183.1 were the two most highly up-regulated transcripts and are good candidates for qualitative expression markers for *Lr34/Yr18*. Although diagnostic DNA markers are available for *Lr34/Yr18* (4,25), expression markers could be very useful for comparing different slow rusting resistance genes or identifying superior alleles. The utility of these candidate expression markers needs to be tested with different cultivars, developmental stages, and times after inoculation.

An important question is whether these abiotic stress-related signature transcripts are associated with *Lr34/Yr18* rather than some other experimental stress factor. Plants were constantly well-watered and temperature was controlled and constantly monitored, so it is unlikely that they experienced any significant environmental stress. Lin et al. (28) reported that the light mineral oil used in rust inoculations induced expression of three thaumatin-like proteins in oats. However, in our experiments, thaumatin-like proteins and other PR proteins were not up-regulated in mock-inoculated tissues (Table 2). In any case, the abiotic stress signature genes were up-regulated mainly in leaf tips of resistant plants but not susceptible plants or resistant plant leaf bases. If a common experimental stress factor had a direct effect on abiotic stress gene expression, we would not expect to see expression only in resistant tips.

One possible explanation for the abiotic stress signature in resistant leaves is drought or osmotic stress associated with spontaneous tip necrosis. There are two reasons why this seems unlikely. First, we were careful to trim all necrotic and adjacent chlorotic tissues from leaves, so samples contained only healthy appearing tissues. Second, levels of biosynthetic transcripts for ABA, the key osmotic- and drought-stress responsive plant hormone, were not significantly changed in the resistant plants. NCED (9-cis-epoxycarotenoid dioxygenase) is an enzyme in the pathway for biosynthesis of ABA and is strongly implicated as the major rate-limiting step in leaves (44). NCED transcript levels are highly up-regulated by drought stress in both dicots and monocots (44,54). NCED belongs to a multigene family and different members may play different roles in different tissues. NCED is well represented on the Wheat Genome Array, with 12 NCED unigenes from various abiotic and biotic stress libraries from leaves, spikes, kernels, and roots. However, none of these NCED transcripts was significantly up-regulated in resistant plants (Table 2). While this suggests that drought/osmotic stress is not present in the resistant leaf samples, further experimentation is needed to verify this inference.

TABLE 3. Genes down-regulated in resistant isolines with *Lr34/Yr18* in both Thatcher and Jupateco backgrounds

Probe set (unigene) designation[a]	Jupateco mock-inoculated		Thatcher mock-inoculated		Thatcher rust-inoculated		Description
	Tip[b]	Base	Tip	Base	Tip	Base	
Ta.11025.1.A1_at		−1.6[c]		−1.5			CPRD2-like FAD-linked oxidoreductase
Ta.12091.1.S1_at	−1.6	−1.6	−1.7	−1.5			Predicted protein
Ta.13337.1.S1_at		−1.7		−2.1		−3.2	Xyloglucan endotransglycosylase
Ta.13337.2.S1_at		−2.7		−4.4		−4.2	Xyloglucan endotransglycosylase
Ta.24639.2.S1_at		−1.9		−2.4	−1.7	−1.9	Predicted protein
Ta.7843.3.S1_x_at		−2.3		−1.6			No homology

[a] Probe set designations from Affymetrix.

[b] Tip indicates the apical half of the flag leaf during anthesis; base indicates basal half.

[c] Indicates the fold change in resistant lines compared with their respective susceptible isolines. Fold change for each cultivar background, inoculation treatment, and leaf tissue was calculated separately with three replicates. Only changes of ≥1.5 fold and $P \leq 0.05$ are shown.

TABLE 4. Fold changes in gene expression induced by rust infection in leaf tips and bases of resistant and susceptible Thatcher isolines 48 h after inoculation

Probe set (unigene) designation[a]	Thatcher-*Lr34* resistant		Thatcher susceptible		Description
	Tip[b]	Base	Tip	Base	
Up-regulated					
TaAffx.83275.3.S1_s_at	21.9[c]	10.8	13.8	11.4	PR-1
TaAffx.83275.1.S1_at	33.0	10.7	11.2	9.7	PR-1
Ta.27327.1.S1_x_at	20.7	9.3	12.2	8.1	PR-1
TaAffx.31943.1.S1_at	16.0	6.5	7.9	4.3	PR-1
TaAffx.119315.2.S1_x_at	12.8	5.4	10.5	3.8	Beta-1,3-glucanase (PR-2)
Ta.8584.1. S1_at	48.7	20.7	8.9	4.0	Beta-1,3-glucanase (PR-2)
TaAffx.24475.1.S1_x_at	35.2	14.1	9.9	5.3	Beta-1,3-glucanase (PR-2)
TaAffx.24475.1.S1_at	36.7	15.8	9.4	6.3	Beta-1,3-glucanase (PR-2)
TaAffx.108743.2.S1_at	14.6	5.2	8.0	3.2	Beta-1,3-glucanase (PR-2)
TaAffx.107478.1.S1_at	14.2	6.4	7.6	5.7	Beta-1,3-glucanase (PR-2)
TaAffx.107478.1.S1_x_at	15.0	6.9	6.9	4.9	Beta-1,3-glucanase (PR-2)
TaAffx.15327.1.S1_at	10.4	6.0	5.4	2.4	Beta-1,3-glucanase (PR-2)
Ta.2278.1.S1_x_at	17.3	7.9	7.1	3.5	Chitinase (PR-3)
Ta.2278.2.S1_a_at	18.3	9.0	8.5	3.5	Chitinase (PR-3)
Ta.2278.2.S1_x_at	17.6	7.4	8.1	3.5	Chitinase (PR-3)
Ta.2278.3.S1_x_at	26.8	12.5	12.1		Chitinase (PR-3)
TaAffx.108556.1.S1_at	21.9	8.7	4.9		PR 4
TaAffx.116570.1.S1_s_at	62.3	23.0	11.5	6.5	PR 4
TaAffx.116570.1.S1_at	49.3	16.2	10.8	5.8	PR 4
TaAffx.79273.1.S1_at	17.8	8.6	9.4	3.1	PR 4
Ta.9226.1.S1_at	9.9	8.7	5.2	2.5	PR 4
Ta.25053.1.S1_at	10.2	8.5	7.0	3.9	Thaumatin-like (PR-5)
TaAffx.108908.1.S1_x_at	11.9	5.3	6.9	5.1	Thaumatin-like (PR-5)
Ta.959.1.S1_at	16.2	7.6	6.2	4.0	Thaumatin-like (PR-5)
TaAffx.128418.43.S1_at	35.7[c]	13.1	11.2	5.3	Chitinase
Ta.22678.2.S1_a_at	12.2	4.8	5.2	2.6	Chitinase
Ta.6051.1.S1_a_at	13.1	6.4	5.1	2.8	Chitinase
Ta.2784.1.A1_at	14.2	7.2	6.6	2.8	Chitinase
Ta.21342.1.S1_x_at	10.7	13.4	6.4	3.1	Chitinase
Ta.97.2.S1_x_at	14.9	6.7	6.8	3.2	Wheat WIR1A homolog
Ta.97.1.S1_at	14.3	5.9	5.8		Wheat WIR1b
TaAffx.108353.1.S1_at	142.8	28.5	10.7	6.7	Naringenin-chalcone synthase
TaAffx.108314.1.S1_at	45.2	17.2	8.5	4.0	Caffeic acid O-methyltransferase
Ta.27338.1.S1_at	48.4	18.4	7.2	3.2	Caffeic acid O-methyltransferase
TaAffx.81099.1.S1_at	38.8	18.6	7.8	3.7	Cycloartenol synthase
Ta.8674.1.A1_at	39.7	15.9	7.8	3.7	Cycloartenol synthase
TaAffx.113333.2.S1_at	38.1		8.4	6.5	O-Methyltransferase
Ta.5810.1.S1_at	29.8	11.8	46.5	7.7	Wali6/Wali3-like
TaAffx.132498.1.S1_at	6.1	8.7	9.6	8.4	Protease inhibitor
TaAffx.55315.1.S1_at	10.5	5.1	5.3		Protease inhibitor
TaAffx.26875.1.S1_at	25.7	17.5	9.1	6.4	Cytochrome P450
TaAffx.53952.1.S1_at	52.7	15.2	8.3	4.8	Cytochrome P450
Ta.22615.1.S1_at	32.6	14.3	6.6	3.5	Cytochrome P450
Ta.30739.2.S1_at	13.0	5.3	5.9	3.6	Cytochrome P450
Ta.8390.1.S1_at	10.1	8.0	5.6	2.5	Cytochrome P450
TaAffx.107587.1.S1_at	23.9	9.3	4.0	5.4	Cytochrome P450
Ta.24710.1.S1_at	68.8	13.0	7.0		Peroxidase
Ta.25845.1.S1_at	24.1	5.4	7.6	5.7	Sucrose phosphatase
TaAffx.21593.1.S1_at	15.4	7.5	10.5	8.2	Aminotransferase
TaAffx.108909.1.S1_at	17.3		6.9	8.5	Aminotransferase
TaAffx.108909.1.S1_s_at	16.6	7.2	6.3	8.0	Aminotransferase
Ta.2690.1 S1_at	5.5	9.9	5.2	2.3	Glutamine-dependent asparagine synthetase
Ta.1745.1.S1_at	5.3	5.2	6.4	5.3	Oxidoreductase
Ta.15159.1.S1_at	19.5	5.7	6.5	2.9	Predicted protein
Ta.22693.2.A1_at	24.6		15.6	46.9	Predicted protein
TaAffx.54541.1.S1_at	27.5	8.3	20.4	13.5	No homology
Ta.23219.1.A1_x_at	14.0	3.7	12.9	9.1	No homology
Ta.413.1.S1_at	23.0	11.7	12.9	5.3	No homology
Ta.14903.1.S1_at	93.6	19.4	8.5	4.4	No homology
TaAffx.28836.1.S1_at	6.6	12.5	6.9	5.3	No homology
TaAffx.113758.1.S1_at	19.6	12.1	3.5	7.9	No homology
Ta.23327.1.S1_at	8.8	2.6	8.2	5.2	No homology
Ta.8653.1.S1_at	8.6	5.7	5.7	3.7	No homology
TaAffx.28836.1.S1_at	6.6	12.5	6.9	5.3	No homology
Down-regulated					
Ta.29534.1.S1_x_at	−8.2	−21.2	−5.1	−4.0	Cysteine proteinase
TaAffx.100446.1.S1_at	−5.9		−5.9	−5.0	ACC oxidase/ethylene-forming enzyme (ACO)

[a] Probe set designations from Affymetrix. Data include two probe sets for the same predicted gene for Ta.2278.2, TaAffx.116570.1, TaAffx.24475.1, TaAffx.107478.1, TaAffx.108909.1.

[b] Tip indicates the apical half of the flag leaf during anthesis; base indicates basal half.

[c] Fold difference increase in expression when inoculated tissue is compared with uninoculated tissue. All changes were significantly different ($P < 0.05$) with at least a fivefold increase in estimated transcript in at least three of four tissues (basal or apical leaf halves of Thatcher or Thatcher-*Lr34*).

Another possible explanation for the abiotic stress signature in resistant leaves is some type of signaling or regulatory mutation that results in ectopic expression of a large set of abiotic stress response genes. Abiotic stress genes are regulated by several types of stress-responsive *cis*-acting elements including ABA-responsive elements (ABRE), cold- and dehydration-responsive elements (DRE), NACR, MYBR, MYCR, and several others (61). We identified three up-regulated transcription factors in resistant plants, including one NAC transcription factor (TaAffx.122104.1) and one zinc finger protein (TaAffx.98394.1). NAC transcription factors and zinc finger proteins can be dehydration- and osmotic stress-responsive in *Arabidopsis* (61). Other potential signaling or regulatory proteins included three LTPs and a protein phosphatase. Any of these could be involved in generating the abiotic stress signature either upstream or downstream in a signaling cascade. On the other hand, it is possible that no transcriptional change would be observed for a key regulatory protein if the protein was regulated posttranscriptionally or a mutation altered its function or activity.

In order to focus on genes with a possible functional role in resistance, we reasoned that key transcripts should be significantly up-regulated or down-regulated in both the tips and the bases of resistant leaves. Therefore, we shortened the list of genes in Table 2 by requiring that genes be up-regulated in resistant plants in both the mock-inoculated leaf tips and bases. Only five transcripts fit these criteria.

Ta.6567.3 (Fig. 2C) has homology to *Arabidopsis* drought-induced protein Di19 and cotton ovule fiber protein Fb2. Gosti et al. (17) reported that Di19 responded to drought in an ABA-independent manner. The function of this protein is unknown. It did not maintain the correlation between expression and resistance in the rust-inoculated plants, which suggests it may not be a key part of the resistance response.

Ta.14836.1 (Fig. 2D) is of interest because it is the most highly and consistently up-regulated gene in resistant leaf bases, both pre- and post-challenge (Table 2). The sequence for this gene matched nothing in sequence databases. TaAffx.64112.1 showed a modest but consistent up-regulation in resistant leaf tips and bases. It has high homology to a rice predicted protein, but the function is unknown. More work is needed on these two proteins.

Ta.28327.1 (Fig. 2E) was consistently expressed more highly in resistant leaf tips than in bases, which mirrors the *Lr34/Yr18* resistance phenotype. It is a type 2 nonspecific LTP that is well-characterized structurally (9), but little is known of its biological role. A closely related barley homolog is aleurone-specific (20). LTPs mediate the transfer of phospholipids between membranes and may have a role in vesicle fusion and secretion. LTPs are involved in many signaling processes (3) and certain nonspecific LTPs have been shown to affect pathogenic microbes when expressed in transgenic plants or in vitro (37,39). Many LTPs are pathogen-inducible and have recently been included in the PR proteins as a new class, PR-14 (57). Another two LTPs (Ta.13070.1 and Ta.13070.2) were up-regulated by *Lr34/Yr18* only in leaf tips. They are homologous to a family of type 1 nonspecific LTPs from barley that are expressed in a variety of tissues, including the spike, and the expression of some of these genes is affected by cold treatment and pathogen infection (34, 35). Transcripts of very similar LTPs were found to be increased by both salicylic acid and methyl-jasmonate application to young wheat seedlings (29). Considering the variety of possible functions of these proteins and their expression patterns, LTPs are good candidates for a functional role in resistance mediated by *Lr34/Yr18*.

Ta.13183.1 (Fig. 2F) was one of the most highly up-regulated transcripts in leaf tips as observed with two different probe sets for this predicted gene, Ta.13183.1.S1_s_at and Ta.13183.1.S1_x_at. The first probe set detected no significant *Lr34*-regulated differences transcript levels in leaf bases (Fig. 2F,

Table 2) while the second probe set detected only small (less than twofold) differences in leaf bases. The predicted gene has very high identity with a cold-regulated protein in wheat (WCor18) and barley (tma-ap3). It is a member of the highly conserved phosphatidylethanolamine binding protein family, members of which are associated with control of flowering time in both dicots and monocots (6). Considering the possible involvement in developmental signaling and the large up-regulation of this transcript, it is another good candidate for a functional role in the resistance conferred by *Lr34/Yr18*.

Bossolini et al. (4) used a comparative mapping approach to identify candidate genes in the *Lr34/Yr18* region. They speculated that pectate lyases might play a central role in resistance conferred by *Lr34/Yr18* by modifying the cell wall. We did find large transcriptional up-regulation of some putative cell wall modifying enzymes such as xyloglucan endotransglycosylase (Ta.13337.1 and Ta.13337.2) and β-D-glucan glucohydrolase (Ta.27751.5). Xyloglucan endotransglycosylase deserves special mention for the significant down-regulation in resistant leaf bases (Table 3), which suggests both positive and negative regulatory signals associated with resistance. In any case, we did not find any significantly and consistently up-regulated pectate lyase transcripts in resistant plants. This does not eliminate pectate lyase as a candidate gene since we were not able to verify that the specific candidate gene is represented on the wheat array. Likewise, we did not find expression changes in β1-proteasome subunits, which were speculated to be involved with resistance conferred by *Lr46/Yr29* (41). In this case, we were able to verify that a β1-proteasome subunit gene (Os06g04800) identified by Lagudah et al. (25) from the orthologous rice region (chromosome 6S) was represented on the wheat array (Ta.3303.1).

The broad spectrum of resistance and lack of race specificity of *Lr34/Yr18* are reminiscent of SAR. SAR is a form of induced resistance that is associated with elevated expression of typical PR proteins prior to local challenge by a pathogen (11,57). Although the defense function of each PR protein has not been clearly established, it is thought that SAR operates through the joint action of multiple PR proteins (11,57). SAR can be induced by pathogen attack in another part of the plant or by chemical inducers such as salicylic acid (SA) or BTH (11,16,29). If *Lr34* somehow induced the expression of proteins associated with SAR prior to pathogen challenge, this could explain the nonspecific resistance observed, but this did not appear to be the case. In our experiments, typical PR proteins were not significantly up-regulated in mock-inoculated resistant leaves (Fig. 2I to L). It was only after inoculation that PR genes were up-regulated in both Thatcher and Thatcher-*Lr34* (Table 4). In addition, the expression levels of wheat chemically induced genes associated with SAR (16) were not up-regulated in mock-inoculated lines with *Lr34/Yr18* (Table 2).

Induced systemic resistance (ISR) is similar to SAR in that it confers broad spectrum resistance to pathogens (5,58). It is inducible by plant growth promoting rhizobacteria or by application of chemicals such as β-amino-butyric acid (55). ISR is characterized by the expression of jasmonic acid and ethylene-responsive defense proteins after pathogen challenge (5,57). However, ISR is not associated with changes in gene expression prior to local challenge (58). This differs from our results in which we found large changes in gene expression patterns without pathogen challenge (Table 2). However, it is still possible that *Lr34/Yr18* has a role in defense gene priming similar to ISR. After rust inoculation, both resistant and susceptible flag leaves expressed essentially the same set of PR proteins (Table 4), but expression was often higher in resistant leaves (Fig. 2J to L). This is exemplified by several related PR-3-type chitinases (Ta.2278.1, Ta.2278.2, and Ta.2278.3) that were all up-regulated two- to threefold higher in inoculated resistant leaf tips and bases compared with susceptible leaves (Fig. 2J). Other transcripts showed

patterns of enhanced expression only in the resistant leaf tips (Fig. 2K and L). As a group, these defense gene expression patterns correlated well with the resistance phenotype associated with *Lr34/Yr18*. Further experiments using multiple time points are needed to test this priming hypothesis.

Although the objective of this study was to characterize *Lr34/Yr34*, this study also is an important contribution in identifying wheat genes that are differentially regulated by rust infection. While rust-inoculated material was only examined from the Thatcher isolines, comparisons of inoculated to mock-inoculated in both halves of the flag leaves and both the Thatcher-*Lr34* and Thatcher lines should provide a reliable list of genes induced by rust inoculation (Table 4). Examples include many genes encoding typical PR proteins, enzymes involved in phenylpropanoid biosynthesis, and enzymes involved in detoxification of various metabolites.

Two genes were highly down-regulated by rust infection. One gene showed homology to cysteine proteinases (Ta.29534.1.S1_x_at). The most similar proteinases in GenBank for which functions have been associated are involved in senescence (1,36). The other down-regulated gene (TaAffx.-100446.1.S1_at) was predicted to code for ACC oxidase, an enzyme involved in ethylene biosynthesis. More information is required on the regulation of these genes to determine whether their down-regulation is an outcome of host defense signaling or specifically regulated by the leaf rust fungus. The inhibition of these proteins by the pathogen may inhibit cell death or senescence and contribute to the classic "green island" effect surrounding pustules in a susceptible interaction.

The present study provided an initial picture of the function and molecular effects of an important adult-plant, durable, slow rusting gene that has become widely used. Since the gene expression patterns associated with *Lr34/Yr18* do not fit typical *R*-gene mediated defense responses or previously described disease resistance pathways, it appears that *Lr34/Yr18* has a novel mode of action involving increased expression of a set of abiotic stress or seed maturation genes not usually associated with pathogen defense reactions. The enhanced expression of one or more of these gene products may directly make the leaf more resistant to rust fungi. Another possibility is that these genes create an environment in the leaf in which the typical defense responses to rust infection are primed and occur more strongly or rapidly after infection. An unexpected finding was the vast difference in gene expression pattern between leaf tips and bases. A comprehensive model of the mechanism of *Lr34/Yr18* will have to explain that difference.

One important use of the characteristic signature of *Lr34/Yr18* will be in comparisons with other slow rusting genes. Genes like *Lr46/Yr29* are phenotypically very similar to *Lr34/Yr18* (41,52) and may share similar modes of action. The *Sr2* adult-plant stem rust resistance gene also shares some similarities such as durability, race nonspecificity, and association with a necrotic symptom in unchallenged tissue (24). *Lr34/Yr18*, *Lr46/Yr29*, and *Sr2* have been incorporated in many wheat varieties targeted for the developing world by the International Maize and Wheat Improvement Center (CIMMYT, Mexico, D.F.) and are also present in many cultivars grown in developed countries (13,24,51). The increasingly widespread dependence on these slow rusting genes raises concerns whether we may become overly dependent on one mechanism of resistance.

Another important benefit of a better understanding of the mechanism of resistance of *Lr34/Yr18* is the opportunity for fine tuning the resistance response. The list of genes up-regulated in plants with *Lr34/Yr18* is long. It is possible that a small subset of those genes is determinative for resistance and the others are unnecessary or even harmful. Singh and Huerta-Espino (49) documented a 5.9% lower grain yield in Jupateco 73R than in Jupateco 73S when rust was controlled with fungicides. A portion of this yield decrease could be due to leaf tip necrosis or other side effects of the resistance conferred by *Lr34/Yr18*. It might be possible to eliminate or reduce the unwanted negative side effects of *Lr34/Yr18* by down-regulating particular dispensable genes. It might also be possible to increase the effectiveness of *Lr34/Yr18* by altering the expression of particular essential genes.

ACKNOWLEDGMENTS

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